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DDE IN BIRDS' EGGS: COMPARISON OF TWO METHODS FOR ESTIMATING CRITICAL LEVELS

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Since the classic study of Ratcliffe (1967) that demonstrated a decrease in eggshell weight of raptors after World War II in Great Britain, numerous studies have been conducted to determine the critical value of change in eggshell quality that will result in a serious population decline if that value is consistently equaled or exceeded over a number of years (see review by Cooke 1973). Hickey and Anderson (1968) determined that eggshell thinning of 18–26% was associated with decline or extirpation of populations of three raptorial species in the United States. The eggshell problems coincided with the widespread use of DDT after World War II, and experiments with captive Mallards (*Anas platyrhynchos*) and later with American Kestrels (*Falco sparverius*) verified that dietary DDE (a metabolite of DDT) induced eggshell thinning and reproductive impairment (Heath et al. 1969, Wiemeyer and Porter 1970, Lincer 1975).

One method for calculating the critical value of DDE in birds' eggs involves use of regression analysis; this residue value corresponds with the predetermined critical value of eggshell thinning that is associated with marked declines in other populations (Keith and Gruchy 1972, Pearce et al. 1979, Fox et al. 1980). A second method—the sample egg technique—provides a more direct measure of the critical residues of DDE and other pollutants that adversely affect nest success (Blus et al. 1974, Blus 1982, Henny et al. 1983).

The purpose of this paper is to compare and evaluate the critical values of DDE that are derived from these two methods using the Brown Pelican (*Pelecanus occidentalis*) and the Black-crowned Night-Heron (*Nycticorax nycticorax*) as the primary models.

METHODS

Brown Pelican relationships were derived from analyses of 813 eggs that were collected from 1969–1976 in California, Florida, Louisiana, and South Carolina (Blus 1982). The Black-crowned Night-Heron study involved 220 eggs that were collected from 1978–1980 in Nevada, Oregon, and Washington (Henny et al. 1983). The regression method depends on the derivation of critical values of eggshell thinning and DDE residues in eggs. A critical value of thinning is a mean calculated from a sample of eggs collected in some time interval from a population that experienced a marked decline or extirpation. Using this method, the critical residue of DDE for a particular population or species is derived from regression analysis; the critical value of thinning—≈18–20%—is then used to predict the critical residue of DDE (Keith and Gruchy 1972, Pearce et al. 1979). A test for sample size (Sokal and

Rohlf 1969:247) is recommended for determination of the number of residue analyses that are required for a statistical assessment of the mean DDE level in a sample population in relation to the critical level.

The sample egg technique was apparently used initially by Ratcliffe (1967); it involves contemporary collection of one egg from each nest in a series within a population. Nests are marked and the fate of the nest is determined. Eggs are analyzed for residues of DDE and other organochlorines, and if appropriate, for other pollutants such as certain heavy metals. Residues of each pollutant are arranged from lowest to highest, and success of each nest is then compared directly with residues in the representative egg. Effects of DDE or other residues on nest success are indicated when rate of success decreases with an increase in residues.

Determination of effects of DDE or other residues in sample eggs as related to success of individual nests is relatively straightforward; whereas extension of these results to predict effects of residues on reproductive success and status of an entire population is much more difficult. The first step is to determine a critical value of DDE or other pollutants in sample eggs; this is the lowest value that results in a marked decrease in productivity and is likely to induce a serious decline in a population in which that value is frequently equaled or exceeded. It is also useful to determine the lowest residue value that is associated with total reproductive failure.

After sampling an adequate number of eggs as outlined for the first method (Sokal and Rohlf 1969:247), the mean residue for the population is compared to the critical value. If the mean residue falls into the critical range, then the population is likely to be severely affected. Another statistical test for evaluating reduction in productivity involves calculation of the percentage of nests with sample eggs that contain critical residues. This is an important consideration because it is conceivable that the population could be affected by pollutants even though the mean residue is less than the critical value determined from individual eggs.

Contents of the eggs were analyzed individually for residues of organochlorine pesticides, their metabolites, and polychlorinated biphenyls (PCBs); analyses were completed at the Patuxent Wildlife Research Center, Laurel, Maryland. Samples were analyzed by electron-capture gas chromatography or thin-layer chromatography; residues in some samples were confirmed with use of a combined gas chromatograph/mass spectrometer. The lower limit of detection was 0.5 μg/g for the PCBs and 0.1 μg/g for the other organochlorines; residues are expressed on a fresh wet weight basis (Blus et al. 1977, Henny et al. 1983).

RESULTS

Regression analysis indicated that DDE was significantly related to eggshell thinning in Brown Pelicans ($R^2 = 0.267$, $P < 0.00001$; Fig. 1) and Black-crowned Night-Herons ($R^2 = 0.312$, $P < 0.001$), although there tended to be a wide range in eggshell thickness with a given residue level. An important factor in this relationship is the inherent variability in eggshell thickness of eggs of birds (Anderson and Hickey 1970, Klaas et al. 1974). The calculated critical threshold of DDE that is associated with 20% eggshell thinning is about 8 μg/g for the pelican and 54 μg/g for the heron. There is some evidence that the critical value for eggshell thinning may be as low as 18% in some populations (Hickey and Anderson 1968);

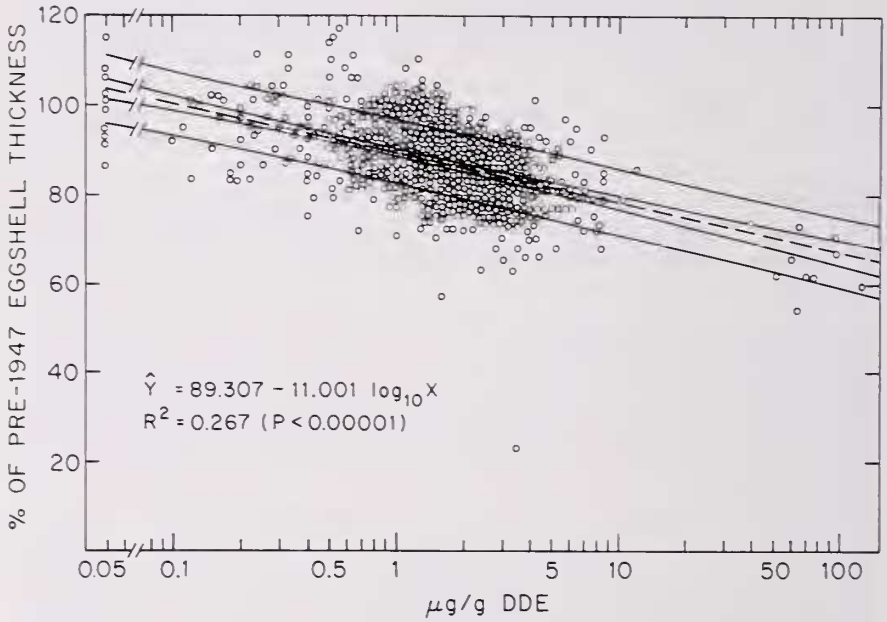

FIG. 1. Regression analysis showing the relationship of DDE residues in 813 eggs of Brown Pelicans to eggshell thickness; South Carolina, Florida, Louisiana, and California, 1969–1976. The dashed line is the regression line, the two pairs of solid lines delineate the 95% confidence limits for the population mean (inner pair) and for individual eggs (outer pair).

the critical values for DDE derived from 18% thinning are 5 μg/g for the pelican and 36 μg/g for the heron.

Using results of the sample egg technique (Fig. 2), mean nest success of Brown Pelicans arrayed by μg/g intervals of DDE ranged from 30–50% through 3 μg/g. Success was lower than expected (30%) at the ND–1 μg/g interval—probably an artifact related to the small sample size. Success was highest (50%) in the 1–2 μg/g interval and declined only slightly to 42% in the 2–3 μg/g interval. Notably, success of nests with sample eggs containing from 2.6–3.0 μg/g decreased by approximately 40% to 29% and declined precipitously above 3 μg/g; total reproductive failure occurred when DDE residues exceeded 3.7 μg/g. Thus, the critical value is 3 μg/g, that is, the lowest level of DDE that would result in severely lowered reproductive success and population decline or extirpation if it prevailed through most of the breeding population for several years.

Use of the sample egg technique for Black-crowned Night-Herons also indicated adverse effects of DDE residues on nest success. Nest success

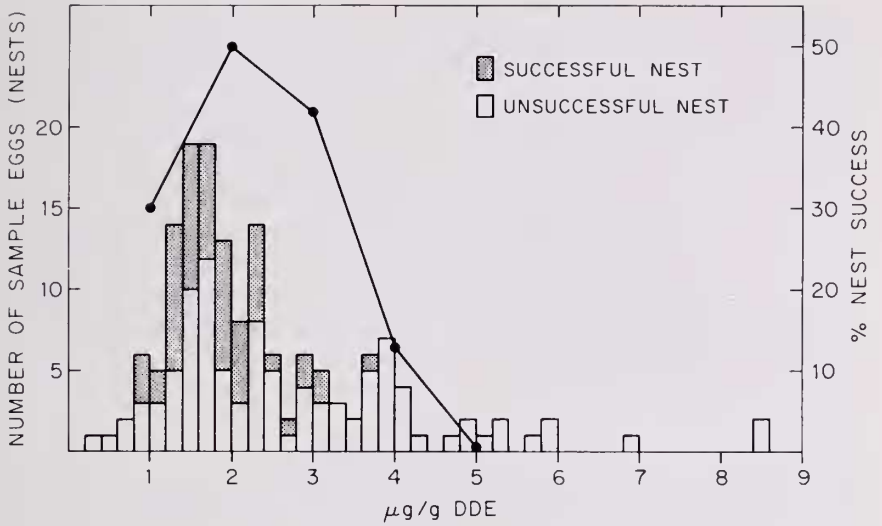

FIG. 2. Relationship of DDE residues in 156 sample eggs of Brown Pelicans to nest success. Bars represent success related to 0.2 μg/g intervals; dots on the line represent mean nest success by μg/g intervals.

ranged from 73–79% when eggs contained ≤ 8 μg/g; however, it decreased 27–58% when sample eggs contained from 8–12 μg/g. Total reproductive failure occurred only when DDE residues ranged from 25–50 μg/g. The critical level of DDE in the heron is near 12 μg/g, although the effects of DDE on nest success are extended over a wide range of residues in contrast to the precipitous decrease in success of Brown Pelican nests. The extended effects of DDE on nest success for the night heron resembled that defined for the Merlin (*F. columbarius*) in Canada (Fox 1979).

The ratio of estimated critical values of DDE that is derived from the two methods, assuming a critical thinning value of 20%, is ≃2.5:1 for the pelican and ≃4.3:1 for the heron; the respective ratios assuming a critical thinning value of 18% are ≃1.6:1 and 3.0:1. Using the critical DDE levels determined from the sample egg technique, the predicted mean eggshell thinning as derived from the regression equation is 16% at 3 μg/g (pelican) and 13% at 12 μg/g (heron).

Regarding sample size of eggs necessary to establish critical values of thinning or residues, the inherent variability (skewness) of residue data necessitates relatively large sample sizes. Regarding the Brown Pelican, the coefficient of variation (CV) calculated for DDE residues ($\log_{10}$) in a given year and locality (South Carolina) ranged from 39–61%. Using a

mean CV of 48%, power = 0.8, and $\alpha = 0.05$, 100 samples are required to be 80% certain of detecting a 20% difference between the sample mean and the critical value at the 5% level of significance. As the certainty of detection declines to 75 or 70%, the number of samples required decreases to 50 and 40, respectively. For the Brown Pelican, 40 analyses per year or over several years when residues are stable probably represents a minimal sample size that would be meaningful from a statistical standpoint. With this number, one could be assured ($P \leq 0.05$) that a residue mean that was equal to the critical value was within 30% (2.10–3.90 μg/g) of that value. From a practical standpoint, lower sample sizes may provide insight into potential problems from DDE and other organochlorines; but reliability of results decreases accordingly.

DISCUSSION

There are several possible explanations for the differences in critical values of DDE in eggs of both Brown Pelicans and Black-crowned Night-Herons, derived from the two methods. There are a number of modes of action through which DDE and other organochlorines directly affect reproductive success; these include a decline in egg production, aberrant incubation behavior, delayed ovulation, mortality of breeding adults, thinning and other deficiencies of eggshells, embryotoxicity irrespective of eggshell deficiencies, and mortality or aberrant behavior of recently hatched young (see review by Blus 1982). Thus, if only effects derived from eggshell thinning are considered, other effects are not fully accounted for and the estimated critical value of DDE is probably too high. Another possibility is that the critical value of DDE determined by the sample-egg technique is biased because of the problem of intercorrelation of residues. The organochlorine pollutants tend to be highly intercorrelated in both pelican and heron eggs, such that it is sometimes difficult to assess and quantify effects that are induced by individual pollutants. In the Brown Pelican and Black-crowned Night-Heron studies, this problem was largely ameliorated by statistical analyses and by collection of a large number of eggs from widely scattered geographic locations where there were differences in residue profiles. In deriving critical levels, errors that result from intercorrelations of residues are apparently much less serious than those that arise from the consideration of only those reproductive effects that are induced by the adverse properties of shell thinning.

A third possibility is that the critical value of shell thinning for the pelican and heron are much lower than the 18–20% derived from long-term studies showing serious decline or extirpation of populations (Hickey and Anderson 1968). Lower critical levels of shell thinning have been suggested for certain other species on the basis of short-term studies

(Capen 1977). This seems unlikely for the Brown Pelican because certain populations experience good reproductive success when shells of sampled eggs averaged 17% thinning (Blus et al. 1977).

Relying on eggshell quality as an indicator of reproductive impairment without residue analysis also has the disadvantage of eliminating effects of other pollutants such as endrin (Blus et al. 1979a) and heptachlor epoxide (Blus et al. 1979b) that act adversely on reproductive success in ways that are primarily unrelated to shell thinning. Effects of these insecticides were determined by using the sample egg technique.

Another problem that occurs in determining the critical level of DDE as interpolated from eggshell thinning data is that erroneous interpretations are likely if the regression line is arbitrarily extended beyond the data points (Snedecor and Cochran 1967) into the zone of critical thinning. For example, in studies of the Common Loon (*Gavia immer*), projections of the critical level of DDE in eggs that were related to 20% eggshell thinning ranged from 14 μg/g (Price 1977)–47 μg/g (Fox et al. 1980); residues were probably too low in both studies to adequately define the critical level of DDE. Similar interpretive problems were likely when low residues of DDE in eggs of several species of seabirds from Canada were used to predict a critical residue associated with 20% shell thinning (Pearce et al. 1979). Another example of this problem relates to the Great Blue Heron (*Ardea herodias*) in the Pacific Northwest (Blus et al. 1980). DDE residues and eggshell thinning were too low to determine critical values, but the regression line was arbitrarily extended beyond the data base for comparative purposes. This analysis indicated that a critical value of 19 μg/g of DDE was correlated with 20% eggshell thinning; whereas, the actual critical value of DDE, although not firmly established, is apparently several times higher (Vermeer and Reynolds 1970). Another example of this problem is provided by arbitrary interpolation of a regression equation that was derived from data on Brown Pelican eggs that were collected in Florida in 1969 where mean eggshell thinning was 7.5% (Blus et al. 1972b). In this regression analysis, 39 of 49 eggs contained residues ≤ 3 μg/g and the maximum residue was 6 μg/g. However, arbitrary extension of the regression line predicted a critical value of 36 μg/g that corresponded with 20% eggshell thinning. Thus, errors are likely when interpolating critical values of DDE from regression lines extending beyond the data base; these errors may result in spurious estimates that may either be much higher or lower than the true values. When sufficient eggshell thickness and residue data are available for estimating critical values of DDE from the regression equation, the estimates are meaningful but are likely to be inflated because adverse effects unrelated to eggshell thinning are not taken into account. Also, variability in thickness is too great to permit

accurate prediction of the influence of DDE on success of individual nests except when the residues are either extremely high or low (Fig. 1). For example, the 95% confidence limits for individual eggshell thickness measurements were 77% and 92% of the pre-1947 norm for sample eggs that contained 3 μg/g of DDE—compared to 95% confidence limits of 83–85% for the population mean (Fig. 1). Thus, this technique is primarily restricted to an assessment of the population effect, whereas the sample egg method may be used for predicting success of the individual nest and the population.

Establishing critical levels of pollutants in eggs and tissues of sensitive species of wildlife is a necessary procedure in assessing effects of these chemicals on individuals and populations. Once the correlative evidence from field studies is firm enough to establish a critical level, then collection of the relevant samples and proper chemical analysis is usually all that is required to demonstrate a problem situation. However, experimental interpretation of critical levels should be made when feasible in order to provide further verification of the findings in the field. The sample egg technique, although having certain disadvantages (Blus 1982) is generally more accurate in assessing critical levels of DDE than the other method. It has the advantage of simultaneous assessment of combined effects of persistent pollutants other than DDE and those induced by DDE which are unrelated to eggshell thinning. Despite these obvious differences, both methods are useful and can sometimes be used to complement one another.

SUMMARY

The sample egg technique and eggshell thickness-residue regression analysis were comparatively evaluated as tools in estimating critical levels of DDE in birds' eggs that seriously affect reproductive success and population starts.

In comparing critical values of DDE that were derived from the two methods, the estimates were lower using the sample egg technique for both the Brown Pelican (3 μg/g vs 8 μg/g) and the Black-crowned Night-Heron (12 μg/g vs 54 μg/g) assuming a critical value of eggshell thinning at 20%.

Extension of the regression line beyond the eggshell thickness-DDE residue data base is likely to result in spurious critical values of DDE. When sufficient thickness and residue data are available for estimating critical values of DDE from the regression equation, the estimates are meaningful but are likely to be inflated because adverse effects unrelated to eggshell thinning such as parental behavior and embryotoxicity unrelated to eggshell deficiencies are not taken into account.

Establishing critical levels of pollutants in eggs and tissues is a necessary procedure in assessing effects of these chemicals on individuals and populations of sensitive species. There are inherent difficulties in quantifying the effects of any pollutant on population trends and declines in productivity. The sample egg technique is apparently a more sensitive method for estimating critical levels of DDE, but some subjective interpretation is required for results obtained by both methods.

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